PATENT

UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 98,365-B1)

In re Application of:	
Rodgers et al.	**Examiner: Audet**
Serial No.: 09/772,819	**Group Art Unit: 1654**
Filed: January 30, 2001	**Confirmation: 3008**
For: Method for Accelerating Bone, Cartilage, and Connective Tissue Growth	

DECLARATION OF KATHLEEN RODGERS Ph.D., UNDER RULE 132

Commissioner for Patents
Box **Response**
P.O. Box 1450
Arlington, VA 22313-1450
Dear Sir,

I, Kathleen Rodgers, Ph.D., declare and state:

1. I am a joint inventor of the invention claimed in the above-identified application and am familiar with the specification, claims, and prosecution history thereof.

2. My laboratory has generated additional data, using the methods disclosed in the above-referenced patent application, Specifically, Sprague Dawley rats underwent intramuscular anesthesia with ketamine/rompum and were prepared for sterile surgery by shaving the surgical site and scrubbing with Betadine scrub followed by 70% ethanol. The rat was then placed on a sterile field in a lateral decubitis position facing the surgeon. The shaved legs were then covered with Betadine solution and draped aseptically. A skin incision was performed parallel to the long axis of the right medial diaphysis. The muscle was separated along fascial planes to expose the tibia. A defect of 1.3 mm in diameter was then drilled from the lateral side of the midshaft cortex so that the defect extended from one cortical side to the other, through the bone marrow. Sterile saline (0.9% NaCl) for injection was then used to clean the surgical area of tissue debris and

bone fragments. Either hydron polymer solution (vehicle: 10% Hydron, 60% ethanol, 1% polyethylene glycol polymer) or test peptide in hydron polymer solution was placed in the bone defect to fill the defect with polymer (approximately 0.1 ml of polymer). The incision was closed with 3-0 Vicryl suture using continuous mattress suture. The animals were allowed to recover from anesthesia, given Bupronex for analgesia and allowed free movement, until euthanasia 7 days later.

3. The peptides tested were the following:

Peptide	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Lys1AIII	--	K	V	Y	I	H	P	F
HomoSer3 AIII	--	R	V	HomoSer	I	H	P	F
NorLeu3 AII	D	R	NorLeu	Y	I	H	P	F
Ala4 AIII	--	R	V	Y	A	H	P	F
AIII	--	R	V	Y	I	H	P	F
Lys2 AII	D	K	V	Y	I	H	P	F
HomoSer4 AII	D	R	V	HomoSer	I	H	P	F
NorLeu2 AIII	--	R	NorLeu	Y	I	H	P	F
Leu4 AIII	--	R	V	Y	L	H	P	F

These peptides are in addition to those demonstrated in the application:

AII(1-7)	DRVYIHP--	SEQ ID NO:4
AII	DRVYIHPF	SEQ ID NO. 1
9GD: NorLeu3-AII(1-7)	DR(nor)YIHP--	SEQ ID NO:45

4. By gross observation, the defects that received peptides were more completely filled with new tissue that had begun to calcify. Microscopic evaluation of the tissue sections confirmed the gross observations. The vehicle-treated defects were filled with fibroproliferative tissue that was well vascularized. In the majority of vehicle treated defects, there was no callus formation and little osteoid formation. Occasionally, an osteoblast was observed at the site of injury.

5. In all of the AII-treated animals, there was extensive fibroproliferative and osteoblastic activity and new blood vessel growth. With AII treatment, callus can be seen external to the cortex as well as within the marrow space. The callus, within the marrow space, is composed of richly vascular fibroblastic tissue with peripheral areas of

new bone formation. The new bone is characterized by innumerable, highly active osteoblasts surrounding islands of osteoid formation.

6. Defects filled with peptide 2A (Lys^1-AIII) resulted in a large amount of fibroproliferative activity, callus formation and deposition of osteoid. In three of 5 tibia, the newly healing tissue appeared to displace the old bone. Similarly, defects filled with peptide 41A ($HomoSer^3$-AIII) had extensive callus formation, osteoid formation and fibroproliferative activity. Defects filled with peptide 2B (Lys^2-AII) resulted in a large amount of fibroproliferative activity, and callus formation. Defects filled with peptide 5A were filled with some fibroproliferative activity and osteoid formation. Bones treated with this peptide had extensive callus formation.

7. The remaining peptides (39B [$NorLeu^3$-AII], 22A [Ala^4-AIII], A(1-9), 41B [$HomoSer^4$-AII], 39A ($NorLeu^2$-AIII) and AIII) had reduced activity compared with AII, peptide 2A, 2B, 5A and peptide 41A, but the osteoid development was superior to vehicle control.

8. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity and/or enforceability of the application and/or any patent issuing therefrom.

Dated: February 6 2004 By: Kathleen Rodgers

Kathleen Rodgers, Ph.D.